DEPARTMENT OF THE ARMY
UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE
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REPLY TO
ATTENTION OF:

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29 November 1990

MEMORANDUM FOR SEE DISTRIBUTION

SUBJECT: USAMRICD Technical Memorandum 90-4, Clinical Notes on Chemical Casualty Care

1. This memorandum, the fourth in a series, is intended to provide technical information to health care professionals of the Army, Navy, Air Force and allied nations on the topic of chemical casualty care, in accordance with the postgraduate medical education mission of the Institute. The information is doctrinally consistent and assumes a working knowledge of the tri-service field manual "Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries," Army FM 8-285, Navy NAVMED P-5041, Air Force AFM 160-11, dated February 1990.

2. Unlimited reproduction and distribution of this memorandum is authorized. Readers are requested to submit topics and questions of general interest for future memoranda of this series. The point of contact for this Institute for instructional assistance and support is the undersigned at DSN 584-3393/2230/3276, Area Code (301) 671-3393/2230/3276.

FOR THE COMMANDER:

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USAMRICD TECHNICAL MEMORANDUM 90-4

PYRIDOSTIGMINE

GENERAL

Pyridostigmine has been fielded in the military as a "pretreatment" for nerve agent poisoning. As a result, military physicians and other health care providers are often asked questions for which answers are not readily available in standard publications. Some commonly asked questions are: How does a cholinesterase inhibitor "pretreat" for nerve agent intoxication? How much benefit does it provide? What are the combined effects of the carbamate and a nerve agent? What will it do to normal people? Will it cause problems with military performance? What will it do to people with chronic illnesses or a tendency toward certain disease states? What will it do to those who take certain medications regularly? Should the need arise, what interaction might there be between pyridostigmine and drugs that might be used during anesthesia?

The purpose of this memorandum is to present the available data relevant to each of these questions.

BACKGROUND AND EFFICACY

Pyridostigmine used alone does nothing for a person poisoned by a nerve agent. It does not reduce the effects, it is not an antidote, and in animal studies, it does not significantly change the LD_{50} of the agent; however, when given before poisoning by a nerve agent and when the nerve agent challenge is followed by administration of the current antidotes (atropine and 2-PAMCl), therapy is more effective than it is without the pretreatment with pyridostigmine, i.e., the LD_{50} is raised.

The effectiveness of a carbamate (e.g., pyridostigmine) as a "pretreatment" for nerve agent poisoning because a carbamate attaches to the same active site on the enzyme acetylcholinesterase (carbamylation) as does a nerve agent, and as long as the carbamate is on that site, the nerve agent cannot bind to the enzyme. After a nerve agent attaches, or binds, the agent-enzyme bond is "irreversible" and the enzyme can be replaced only by de novo synthesis. In contrast, the attachment of the carbamate is "reversible," and within minutes to hours (depending on which carbamate) the carbamate spontaneously leaves, or is hydrolyzed from the enzyme ("decarbamylation") leaving the enzyme able to function normally. Thus, the attachment of a carbamate provides temporary "protection" of the enzyme from the nerve agent.

One might expect that tying up, or inhibiting, the enzyme with a carbamate would cause the same effects as inhibiting the enzyme by a nerve agent. This is true if equieffective amounts of the two inhibitors are used, as a large amount of a carbamate will cause the same effects as nerve agent intoxication; however, it has been found that only a small percentage of functional acetylcholinesterase is necessary for normal or near normal functioning in organs (e.g., neuromuscular preparations) or organisms. If this small amount can be temporarily "protected," the efficacy of immediate, adequate therapy is greatly enhanced. In animal studies, carbamylation of about 30% of circulating acetylcholinesterase (red blood cell, or erythrocyte, cholinesterase) corresponds to a great increase in the effectiveness of the antidotes. This degree of inhibition can be produced in normal humans by an amount of pyridostigmine that causes negligible side effects or performance decrements.

One might further expect that if pyridostigmine inhibits part of the acetylcholinesterase, a smaller amount of nerve agent would be needed to produce toxicity. For reasons not well understood, this is not the case. The administration of pyridostigmine before a nerve agent challenge does not change the LD_{50} from that of nerve agent alone. In limited studies using very small amounts of nerve agents, humans pretreated with pyridostigmine had the same or, in many cases, fewer effects than they did without the pretreatment; in no instance were the effects more severe.

Several studies indicate that a carbamate "protects" the active site on the enzyme. In <u>in vitro</u> studies, Koelle demonstrated that physostigmine (another carbamate) protected acetylcholinesterase against phosphorylation by the DFP (an organophosphorous inhibitor, similar to a nerve agent) (1). About the same time, Leopold and McDonald noted that DFP caused prolonged miosis in the eyes of humans. When DFP was administered after physostigmine, the time course of miosis was short, corresponding to that of physostigmine alone, suggesting that DFP did not attach to the receptor sites (2).

In a more recent study, the duration of neuromuscular blockade in intact animals was measured after the nerve agent soman administration in animals pretreated with pyridostigmine and in animals not pretreated. After soman alone (no pretreatment), the blockade was still complete (0% functional) at the termination of the study; in pyridostigmine pretreated animals, the blockade was briefly 0% at 10 minutes after the soman, but had returned to normal (100%) by 30 minutes after soman administration. In the latter instance, the time course of the blockade was that produced by pyridostigmine alone. As part of this study, soman was administered after a small amount of VX (a persistent nerve agent; the VX-enzyme bond is easily reactivatible by an oxime). A few minutes later oxime was administered, and the complete neuromuscular block returned to normal, indicating that the receptor sites had been

occupied by the pre-administered VX, which is easily removed from the enzyme by an oxime, rather than by soman, which is not removed (3). In both of these studies, the investigators noted a species difference. Primates were most sensitive to pretreatment and therapy, guinea pigs next, followed by rabbits and rats.

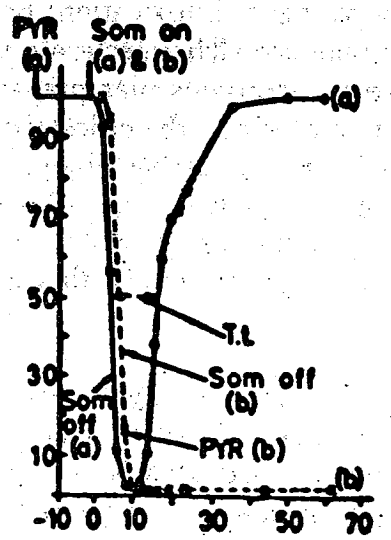

FIGURE. Rhesus monkey soleus muscle. Effectiveness of pyridostigmine pretreatment (100 ug/kg^{-1}, i.v.) in restoring soman depressed tetanus tension. (a) O--O, pyridostigmine (PYR) given 15 min before infusion of soman (14 ug/kg^{-1}, i.v.); (b) x -- x, PYR given 7 min after infusion of SOM (14 ug/kg^{-1}, i.v.). Som: Soman. T.t. tetanus tension recovery time. Ordinate: Tetanus tension (% of maximum). Abscissa: Time (min.)

Pyridostigmine given prior to soman considerably shortened the time of recovery of the soman induced neuromuscular blockade as measured by tetanus tension (curve (a); time course of pyridostigmine) compared to the recovery time when pyridostigmine is given after soman (curve (b); effects of soman (from reference 3).

The first use of a carbamate for "pretreatment" for lethal effects of an organophosphorus compound was in 1946 when Koster (4) reported that the administration of physostigmine before DFP protected cats against an otherwise lethal dose of DFP. Several years later, Wills (5) first used a carbamate as a "pretreatment" for a nerve agent. Atropine was unsuccessful therapy in sarin challenged rabbits (1/5

survived), but when physostigmine was given before the agent challenge and atropine therapy after agent challenge, all five animals survived.

Physostigmine has several drawbacks for field use. Its duration of activity is relatively short (an hour or less) which would necessitate frequent dosing and it causes side effects in man in the dose necessary for protection (physostigmine enters the CNS, and most of these side-effects are CNS related). In the early 1970s, pyridostigmine was found also to be effective. Because of its longer duration of activity dosing intervals are 8 hours; it also causes negligible side effects at the required doses (it does not readily cross the blood/brain barrier).

Efficacy was demonstrated by animal studies. As noted above, there was a species difference in response to pretreatment/therapy. Primates were the most responsive, followed by ginea pigs, rabbits, rats, and mice.

Pyridostigmine pretreatment was most beneficial in the pretreatment of animals challenged by soman (GD), an agent producing an agent-enzyme complex refractory to oxime reactivation. In three studies in guinea pigs challenged with soman and treated with atropine/2-PAMC1 the PR's* were 3.4, 1.7, and 3.0; when pyridostigmine was given before agent challenge and the same therapy given after, the PR's were 6.4, 6.8, and 11.0. In two studies in rabbits, the addition of pyridostigmine pretreatment to the standard atropine/2-PAMC1 therapy raised the PR's from 1.4 and 2.2 to 2.7 and 3.1. In rhesus monkeys, the PR in atropine/2-PAMC1 treated animals was raised from 1.6 to over 40 by the addition of pyridostigmine pretreatment.

After tabun (GA) challenge, the PR's after atropine/2-PAMC1 therapy were 2.4 in rabbits and 4.4 in guinea pigs; pyridostigmine pretreatment raised these to 3.9 and 12.2 respectively.

Standard therapy (atropine/2-PAMC1) given to sarin (GB) and VX challenged guinea pigs produces PR's of 30-50. Pyridostigmine pretreatment did not significantly change these (an observation reported over a decade ago (6)).

*The "protective ratio" (PR) is defined as the ratio of the LD_{50} in a group of animals challenged with an agent and treated to the LD_{50} of a group of animals challenged with an agent and not treated under the same experimental conditions. A PR of 1 would indicate the treatment is of no value. The more effective the treatment is, the higher the PR will be.

In several studies the effectiveness of atropine alone as therapy with and without pyridostigmine pretreatment was investigated, and this effectiveness was compared to that with the addition of an oxime. Generally oximes seem not to assist in the decarbamylation of the carbamate-enzyme complex (they usually are not useful in carbamate poisoning and in some cases may contribute to the toxicity of the carbamate). However, under the conditions of these studies, the addition of an oxime provided a small but definite benefit to the effectiveness of therapy. Harris, et al, suggested that under these circumstances the oxime may prevent recarbamylation of decarbamylated enzyme thereby interfering with the carbamate-enzyme equilibrium, or they may act directly by increasing the rate of decarbamylation of the cholinesterase (7,8).

The time course of the effectiveness of pyridostigmine was investigated in guinea pigs (6). The animals were pretreated with pyridostigmine and an oxime (both given i.m.), given soman after the stated time interval, and then treated with atropine and an oxime. Protective ratios for the times indicated were as follows:

Interval Between Pyridostigmine and Soman	PR
10 minutes	3.4
30 minutes	8.0
60 minutes	12.5
2 hours	6.0
3 hours	4.7
4 hours	3.2

SIDE EFFECTS

Over the past two decades, pyridostigmine (30 mg, in single doses are q.8h. for as long as 2 weeks) has been administered to thousands of military people in several countries. The incidence of side effects has been under 1%, and most were mild and involved the gastrointestinal tract (increased flatus, loose stools). If these effects are severe or prolonged, they might require therapy and they respond well to small amounts of atropine, e.g., one (1) mg i.m. or orally. It is possible that transient muscular fasciculations might also be seen due to the nicotinic effects of pyridostigmine.

Rarely, an individual might be sensitive to the bromide portion of the molecule and develop an erythematous rash.

PHYSIOLOGICAL EFFECTS

In a large number of studies involving hundreds of subjects, pyridostigmine (doses of 30 or 60 mg given once or repeatedly) caused a decrease in heart rate of 5 bpm at rest and on exercise, and no effects on visual parameters including pupil size or "other physiological or blood parameters" (9). Several relevant recent studies are described below.

Five subjects exercised on a bicycle ergometer for 30 minutes (55% peak oxygen consumption) in an environmental chamber; the ambient temperature was 29° C (86°F) and the dew point temperature was 10° C. On one occasion, the subjects took 30 mg of pyridostigmine bromide orally 150 minutes before exercise and on another occasion they took no drug. After the drug, the esophageal temperature was higher ($p<0.01$), the whole body sweating rate was higher ($p<0.01$), the heart rate was lower ($p<0.01$), and the skin blood flow was lower ($p<0.05$). (10).

In a related study, 4 healthy males exercised for 30 minutes (bicycle ergometer; 58% peak oxygen consumption) on 3 occasions without taking pyridostigmine and on 3 occasions 150 minutes after taking 30 mg pyridostigmine orally. Ambient temperatures for these sessions were 22°C (69°F), 29°C (86°F), and 36°C (98°) and the relative humidity was 30%. The findings were similar to those noted above. Compared to the control (exercise alone), pyridostigmine administration and exercise at the higher temperatures caused a small but statistically significant decrease in heart rate, increase in the esophageal temperature, increase in sweating, and decrease in skin blood flow (by 40% at 29°C and by 50% at 36°C). The investigators noted that the decrease in skin blood flow created a less favorable temperature gradient for heat exchange between the skin and the environment, increasing heat storage. They suggested further that, in a chemical protective suit, heat loss by evaporation would be limited by the low water vapor pressure gradient between the skin and the immediate environment, and dry heat loss would become much more important in maintaining body temperature. In such an environment, pyridostigmine might adversely affect temperature regulation and an individual might be more susceptible to heat storage (11).

Seven males took pyridostigmine q.8h. for 4 days and exercised (55 minutes; 40% maximal oxygen consumption; 108°F and 30% r.h.) 2 hours after the first daily dose each day. They underwent the same experimental conditions taking a placebo tablet. There

were no differences between esophageal and skin temperatures, heart rates, and sweat rates between the drug and placebo trials. Skin blood flow was not reported. These findings (lack of differences in heart rates, temperatures, sweat rates between the drug and placebo groups) were in contrast to the differences found in the previous two studies. Although the ambient temperature was higher (108°F vs 98°F and 86°F, the workload was about 30% less (40% maximal oxygen consumption vs 58% and 55%). This suggests that workload, rather than temperature, may contribute more to potential heat storage (Kolka, M. A.; unpublished data). These data suggest that under conditions of moderate to a heavy workload at high temperatures (98°F - 108°F), heat storage with resulting illness may occur; however, the conditions of the third study, in which there were no changes, probably more accurately reflect most military operational scenarios.

Twelve males took pyridostigmine (30 mg q.8h., for four doses) and were subjected to altitudes of 8,000 feet and 13,000 feet, and rapid decompression from 8,000 to 23,000 feet in a simulator. No differences from a placebo trial were noted in PAO_2, SaO_2, $PACO_2$, heart rate, minute volume, forced expiratory volume, forced vital capacity, and forced expiratory flow (12).

PERFORMANCE

In hundreds of subjects in studies conducted over the past two decades, pyridostigmine caused no changes in psychological tests for cognitive and psychomotor skills, memory, manual dexterity and vigilance, and driving tests by day and by night (9). Several studies are described below.

In a double-blind crossover study, 12 subjects underwent testing of sensory, motor, and cognitive functioning at ground level, 8,000 feet, and 13,000 feet after the fourth dose of pyridostigmine (30 mg, p.o., q.8 h.). After pyridostigmine, preferred hand tapping was decreased at 8,000 feet, nonpreferred hand tapping was decreased at 13,000 feet, and memory search recall (short-term memory) was decreased. Although statistically significant ($p<0.05$ in each case), these changes were felt to be operationally insignificant. The investigators concluded that overall performance was not functionally altered by the drug (13).

Twenty one C130 pilots flew a 1.5 hour C131H simulation flight after receiving pyridostigmine (30 mg) or a placebo in a double-blind crossover study. There were no differences noted between the conditions on measures of workload, fatigue, mood, and symptoms, nor were there any differences in overall performance (14).

DRUG INTERACTIONS/CHRONIC ILLNESS

When a threat of exposure to nerve agent has been determined to exist, soldiers may be instructed by their commander to begin taking the pretreatment pyridostigmine (30 mg tablets) every 8 hours. If exposed to nerve agent, the soldier must immediately receive the antidote combination of atropine and pralidoxime by injection to increase the chances of survival. Needless to say, there may be many pyridostigmine-treated soldiers on the battlefield, even though exposure to nerve agent may never occur.

There may be some soldiers with preexisting medical conditions who sometimes require daily medication for adequate control, and yet they may be considered "worldwide deployable." Thus, there may be soldiers subject to a pyridostigmine-drug interaction or a pyridostigmine-medical condition interaction. A possibility of pyridostigmine-drug interaction is based on similar target sites or a resultant change in the pharmacokinetics of either drug. Since there is no significant plasma protein binding by pyridostigmine, this eliminates the consideration of interactions involving competition for these sites. Only 10-25% of pyridostigmine is metabolized, so interactions with biotransformation processes is also of little importance. No attempt will be made to cover potential pyridostigmine interactions with every common drug; however, a few caveats are pointed out with regard to some common chronic medical problems requiring daily medication.

Hypertension: One in six Americans have hypertension. This is a well-recognized medical problem and many successful pharmacologic approaches are used to control this condition. Currently, several classes of drugs are used in the treatment of hypertension: diuretics (thiazide, loop, potassium sparing), beta-blockers, alpha-blockers, alpha$_2$ agonists (primarily centrally acting), converting enzyme inhibitors, calcium channel blockers, and direct acting vasodilators. Of these, few interactions are anticipated.

Beta-blockers, however, may pose a problem. Beta$_1$-antagonists have negative chronotropic and inotropic effects on the heart. Pyridostigmine augments vagal effects on the heart, so additive effects on heart rate may occur, resulting in a further reduction in cardiac output and blood pressure. Non-selective beta blockers also block beta$_2$-receptors and can cause an increase in airway resistance. Bronchoconstriction is not a problem with pyridostigmine at the recommended dosage; however, it may become manifest in an individual who is also taking a non-selective beta blocker or in a previously undiagnosed individual with reactive airway disease.

It is not known if pyridostigmine would increase the incidence of syncope in patients taking alpha-blockers, alpha$_2$ agonists, converting enzyme inhibitors, calcium channel

blockers, or direct acting vasodilators; however, if volume depletion is added to this scenario, this might enhance the likelihood of seeing the additive effects of pyridostigmine with these drugs. Patients should be advised of the possibility of becoming lightheaded, especially if not properly hydrated. Initiation of pyridostigmine under medical supervision should be considered.

Asthma: A known asthmatic is not worldwide deployable. However, a desert environment and/or pyridostigmine may unmask a previously undiagnosed individual with hyperreactive airways.

Glaucoma: Anticholinesterase drugs are often used in the treatment of several forms of glaucoma, so pyridostigmine would not be a problem, but simply additive. Effects of either or both drugs may interfere with night vision. On the other hand, timolol (a non-selective beta blocker) is also used to reduce intraocular pressure, and as mentioned earlier, in combination with pyridostigmine may result in bronchoconstriction in an individual who also has hyperreactive airways.

Low "dibucaine number" or low plasma cholinesterase: Dibucaine is used as a diagnostic tool because it inhibits plasma (or "pseudo-" or "butyro-") cholinesterase to varying degrees. Dibucaine inhibits the normal (homozygote) enzyme by 70-85%, hence a dibucaine #80 indicates the presence of normal enzyme, and the incidence in the population is 96%. Dibucaine #50-65 (50-65% inhibition) is characteristic for the heterozygote with an incidence of about 4%. Dibucaine #16-25 signifies the abnormal homozygote and occurs at a frequency of about 0.03%.

A person with a low dibucaine number has plasma cholinesterase that is resistant to inhibition by dibucaine. Pyridostigmine inhibits normal plasma cholinesterase by about 35%, and although it isn't known exactly how much pyridostigmine inhibits the atypical enzyme in general, the atypical enzyme is also resistant to inhibition by carbamates. However, the purpose of pyridostigmine pretreatment is to protect "true" cholinesterase at the neuromuscular junction (and reflected in red blood cell cholinesterase activity). People with abnormal plasma cholinesterase generally have normal true cholinesterase, and therefore, should be offered the same protection from pyridostigmine.

The significance of a low dibucaine number is that the individual will have a prolonged response to selected drugs, e.g., succinylcholine and ester local anesthetics. This is because the individual has a low quality (not necessarily low quantity) plasma cholinesterase that is normally responsible for metabolizing and inactivating these drugs. Regardless of whether pyridostigmine has an effect on this abnormal plasma

cholinesterase, the individual will have a higher blood level and prolonged response to drugs which require plasma cholinesterases for bioinactivation.

Decreased levels of plasma cholinesterase (as much as 50%) are sometimes seen in people taking birth control pills or corticosteroids, but again, the clinical significance of this in soldiers taking pyridostigmine is not related to the efficacy of nerve agent pretreatment, but rather is confined to enhanced responses to drugs that depend on the enzyme for their metabolism (e.g., succinylcholine and ester local anesthetics).

Antimalarials: Depending on the region, drugs used for prophylaxis/suppression and treatment of malaria symptoms include chloroquine, primaquine, mefloquine, quinine, quinidine, Fansidar (pyrimethamine + sulfadoxine), doxycycline and tetracycline. These drugs act by interfering with parasite replication and protein synthesis; therefore, no mechanistic interactions with pyridostigmine are anticipated. However, there may be interactive side effects: Quinine and quinidine (and possibly mefloquine based on structural similarity) have a weak nondepolarizing blocking effect on skeletal muscle. This side effect would tend to be negated by pyridostigmine. With regard to quinidine's cardiac effects, concurrent pyridostigmine administration may make A-V block more attainable and hypotension accentuated. Another possible interaction between antimalarials and pyridostigmine is the possible additive effects on the gastrointestinal tract. Loose bowels is the most common complaint about both antimalarials and pyridostigmine, and together they may pose a simple inconvenience or possibly a genuine problem.

Gastrointestinal problems (reflux esophagitis, peptic ulcers) may be exacerbated by pyridostigmine.

Hyperthyroid patients may develop atrial fibrillation if administered pyridostigmine.

The enclosed report from Military Medicine provides further information on these topics and on the interactions of pyridostigmine and drugs used in anesthesia.

PHARMACOKINETICS

The pharmacokinetics of oral and intravenous pyridostigmine have been defined in healthy male volunteers and in patients with myathenia gravis(15-18). Pyridostigmine is highly water soluble and the tablet undergoes rapid dissolution. The drug (a quatenary ammonium compound) is a charged molecule, which probably contributes to its variable absorption. Pyridostigmine has a relatively short half-life and is primarily excreted unchanged in the urine. The mean pharmacokinetic parameters for pyridostigmine are summarized in Table 1.

Table 1. Pyridostigmine Pharmacokinetic Data(Ref. 15,17,18)

	Mean	Std. Dev.
Total Clearance(ml/min/kg)	8.5	8.7
Urinary Excretion(%)	80-90%	0.3
Vol.Dist.(L/kg)	1.1	0.3
Half-life(h)		
Intravenous	1.9	0.2
Oral	3.7	1.0

BIOAVAILABILITY

The bioavailability of pyridostigmine has been estimated to be between 14%(17) and 29.1%(15). There is a large intersubject variability in the extent of bioavailability. For example, in one study the mean bioavailability was 29.1% with a range of 14.7-51.1%(15).

PHARMACODYNAMICS

After Single Oral Dosing

There is considerable intersubject variation in the pharmacodynamic effect of pyridostigmine. After oral administration the peak erythrocyte acetylcholinesterase (RBC AChE) inhibition varied from 20 to 39% of the baseline enzyme activity and the period of inhibition exceeding 20% varied from 0.33 to 5.0 hours. The variation appears to be

due to interindividual differences in the amount of drug absorbed, in the rate of elimination of the drug, and in the sensitivity of the RBC AChE to inhibition by pyridostigmine.

After Multiple Dosing

Despite the varability in bioavailability of pyridostigmine and its pharmacodynamic effects following a single dose, relatively stable and predictable inhibition of RBC AChE is produced with multiple dosing. Figure 1 shows the mean RBC AChE inhibition in eight healthy male subjects following doses were given without any particular reference to meals. Using this regimen AChE inhibition was produced in the target range of 20 to 40%.

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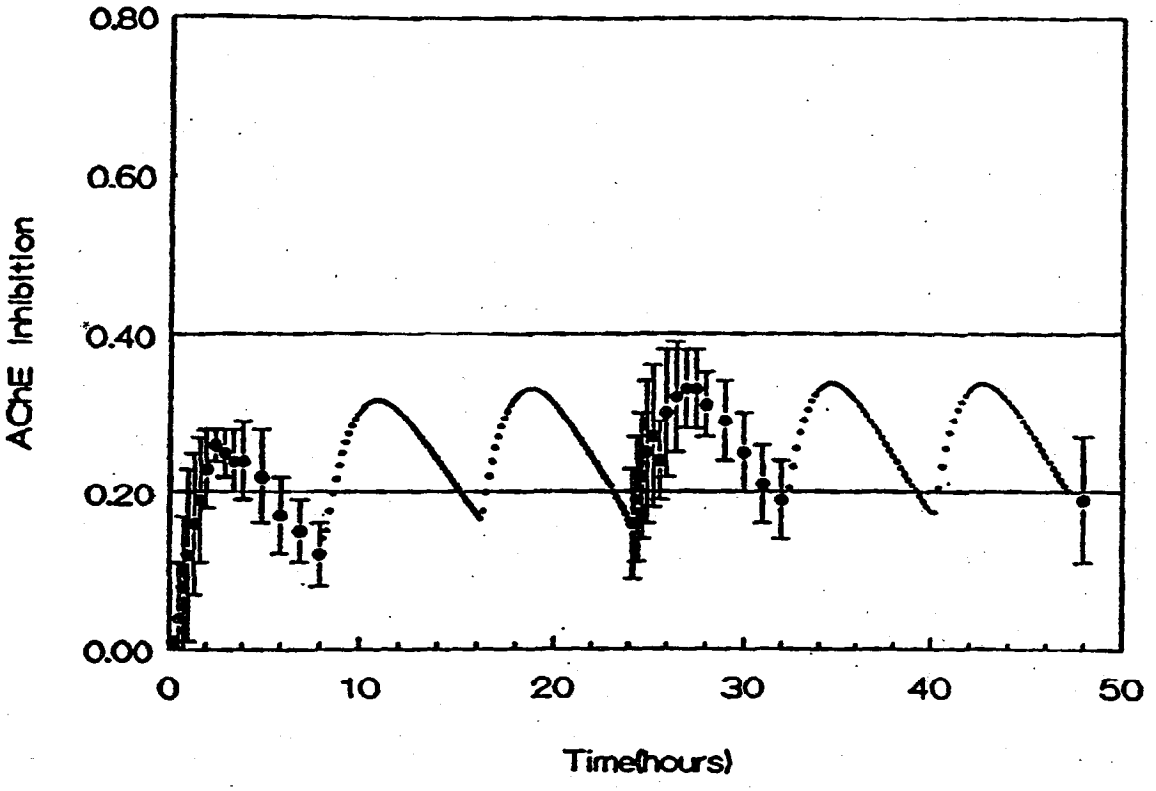
FIGURE 1



LEGEND FOR FIGURE 1. MEAN (+/-STD DEV.) ACHE INHIBITION FOLLOW ORAL ADMINISTRATION OF PYRIDOSTIGMINE (30 MG) TO HEALTHY MALE VOLUNTEERS (N=8) EVERY 8 HOURS FOR SIX DOSES.